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Original Article

Myco-Remediation Potential of Heavy Metals from Contaminated Soil

***[1]O.D. Asiriuwa, [2]J.U. Ikhuoria and [3]E.G. Ilori**
*[1]College of Education, Ekiadolor, Benin City, Edo State
[2]Chemistry Department, University of Benin, Benin City, Edo State
[3]Central Analytical Laboratory, Nigerian Institute for Oil Palm Research, NIFOR, Benin City, Edo State

ABSTRACT
In this study, myco-remediation technique was used to asses the bioaccumulation potential of heavy metal (Cd, Zn, Cu, Pd) by mushroom from heavy metal contaminated soils. Mycoremediation is a form of bioremediation in which various strains of fungi are used to decontaminate contaminated environment. Results obtained from the study revealed that mushroom can bioaccumulate heavy metal from metal contaminated soil. Minimum and maximum concentration of Cu accumulation by the mushroom was 10.60 and 41.80 mg kg^{-1} respectively, with 0 % amendment level accumulating the highest Cu concentration of 41.80 mg kg^{-1}. The bioaccumulation potential of cadmium was the least of the metals in the macro-fungi. Cd concentration ranged from 3.90 to 17.90 mg kg^{-1}. It was observed that the amount of metal accumulation in the mushroom decreased with increased in amount of poultry dropping added to soil.
Keywords: Amendment, Bioaccumulation, Mushroom, Poultry dropping,, Mycoremediation.

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INTRODUCTION

Myco-remediation is a process of using fungi to return an environment (usually soil) contaminated by pollutants to a less contaminated state. It means using various strains of fungi to clean as radio nuclide. Myco-remediation also held promise for removing heavy metals from the land by channeling them to the fruit bodies for removal [1]. One of the primary roles of fungi in the ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extracellular enzymes and acids that breakdown lignin and cellulose, the two main building blocks of plant fiber. The key to myco-remediation is determining the right fungi species to target a specific pollutant.

Mushrooms are a special group of fungi which are saprophytic in their life patterns [1]. They lack chlorophyll and consequently cannot undergo photosynthesis. They feed on organic matter; by producing a wide range of enzymes that can breakdown complex substances after which they are able to absorb the soluble substances so formed. Mushrooms are found in all sorts of environments. They grow on logs (lignicolous), animal dung (coprophylus), agricultural wastes – sawmill dust burnt grounds (phyrophylous), lawns etc. This is why they are good recyclers.

Mushrooms are valuable health foods, low in calories, high in vegetable, proteins, iron, zinc, chitin, fibre, vitamins and minerals [2]. They have been reported to be of therapeutic value, useful in preventing diseases such as hypertension, hyper-cholesterolemia, cancer and also having antibacterial and antiviral properties [3].

Many investigations have dealt with the metal contents of mushrooms, especially edible ones [2 – 4]. Heavy metal concentrations in mushroom are considerably higher than those in agricultural crop plants, vegetables and fruit [5]. This led to suggestion by Turkekul et al; [6] that mushrooms possess a very effective mechanism that enables them to readily take up some heavy metals from the ecosystem.

Mushroom cultivation is a worldwide practice, which utilizes almost all agricultural and agro-industrial residues as substrate. Composted organic material such as plant debris and animal manure add nutrient to the soil thereby increasing the soil fertility. Poultry manure as an organic material is particularly important since it conditions and improves soil fertility and contains all macro-nutrients and most of the micro-nutrients [7].

Mushroom cultivation has become a worldwide practice due to its many uses and application. Interestingly the sclerotia of mushroom are of great importance because they can be preserved and stored for a long time without losing its viability. The sclerotia are also used as food and for medicinal purposes. This article reports research conducted to assess the bioaccumulation of some heavy metal (Cd, Zn, Cu, Pb) planted on metal contaminated soil.

MATERIALS AND METHOD

The soil samples were collected at the top soil surface (0 – 15cm) from a recently cultivated plot of land. Sclerotia of pleutorus tuberrigium were sourced from plant biology and biotechnology department of University of Benin, while the poultry droppings were obtained from a poultry farm in Benin.

PREPARATION OF MATERIAL

Composting the poultry manure

The poultry droppings were composted by heating, resting and tilling until a constant temperature of 30^{0}C was obtained. Physico-chemical analysis of the dung revealed that the dung contain 0.60% N, 0.34 % P, 1.20% Ca, 0.74 % Mg, 0.44% K, 1.00 mg/kg Pb, 0.80 mg/kg Cd, 1.70 mg/kg Cu and 1.00 mg/kg Zn

Substrate Preparation and Cultivation

Composted poultry dropping were added to the soil samples at amended level of 0%, 5%, 10%, 20% and 30% of poultry droppings in four replicates. Each of the amended level replicates was then contaminated with 0.3g of salts of Cd, Cu, Pb and Zn respectively and left to acclimatize for fifteen days. Before planting, the sclerotia after soaking for 2hrs and cutted into sizeable pieces of 20g. The experimental pots were left for 2 weeks but kept moist by addition of water twice a week.

Determination of physico-chemical properties of soil

The physico-chemical properties of the soil; pH [8] particle size [9 – 11], organic carbon [8, 12 – 14], cation exchange capacity [8, 15 – 18], phosphorus [19], nitrogen, nitrate and sulphate, were determined by Standard Methods.

Determination of the total Heavy-Metals in the Poultry Dropping

2g of the poultry dropping were weighed into a 250 mL plastic bottle. 100 mL of 0.1M HCl was added and stoppered and placed on the mechanical shaker for 30mins. The mass then filtered through Whatman filter paper No 42. The heavy metals (Fe, Cu, Mn, Zn, Cd, Cr, Pb) were determined in the filtrate by Atomic Absorption Spectrometer.

Determination of the Total Heavy Metal in the Mushroom/Sclerotia

5 g of each sample of mushrooms were dried in the oven at 105 ^{0}C for 1 hour, 1 g of the dried sample was weighed into a 50 mL pyrex beaker and placed in a muffle furnace set at 500 ^{0}C. The mushroom was left to ash for 3 hrs. 10 mL of the ashed mushroom sample was then dissolved in 20% HNO_3 and heated gently on a hot plate for about 5mins. The sample was allowed to cool and then filtered into 5 mL flask. The flask was then made to mark with water. The heavy metals in the sample were then determined using atomic absorption spectrophotometer.

SEQUENTIAL EXTRACTION OF METALS (5-STEPS METHODS)

Extraction of exchangeable fraction: The exchangeable fraction of the soil was determined by extraction of the metal at room temperature for 1 hr with 8 mL of 1M magnesium chloride solution at pH 7.0

Extraction of Carbonate Bound Metals: The carbonate bound metals fractions was extracted at room temperature with 8 mL of 1M sodium acetate solution adjustment to pH 5 with acetic acid. The mixture was then agitated for 5 hrs

Extraction of Fe-Mn Oxides bound metals: The fraction bound to Fe-Mn oxides fraction was extracted with 20 mL of 0.04M hydroxylamine hydrochloride in 25% acetic acid. Extraction was performed at 96$\pm$ 3 ^{0}C with occasional agitation for 6hrs.

Extraction of organic-bound fraction: To the fraction bound to organic matter fraction was added 3 mL of 0.02M nitric acid and 5 mL of 30% hydrogen peroxide, adjusted to pH 2 with nitric acid. The mixture was heated to 85 $\pm$ 2 ^{0}C for 2 hrs with occasional agitation. An additional 3ml aliquot of the acidified 30% hydrogen peroxide was added and the mixture was heated at 85 $\pm$ 2 ^{0}C for 3 hrs with intermittent agitation. 5 mL of 3.2M ammonium acetate in 20% nitric acid was added to the cooled mixture and the sample was diluted to 20 mL and stirred continuously for 30mins.

Extraction of residual fractions: The residual fraction was digested with a 5ml of aqua regia and 1ml of perchloric acid and diluted to 100 mL with distilled water. After each successive extraction phase, the residue was washed with 10 mL of de-ionized water and the supernatant fraction added to the extract from each step. The concentrations of Cu, Pb, Cd, and Zn in every extract were determined by atomic absorption spectrophotometer [20].

Statistical Analysis

The whole data were subjected to a statistical analysis. The results were expressed as means ± SD of triplicate analysis. Student t-test was employed to estimate the significance of results obtained.

RESULTS AND DISCUSSION

The study showed that Sclerotia of *p.* tuberrigium produced fruity body (mushroom) in the poultry dropping amendment, pots of 0% and 5% levels. The mushroom products were healthy and lively: 10% level of amendment produced weak and dying fruiting bodies. 20% and 30% produced nothing. This result corroborates the findings of Shaikli and Ghoffer [21], Khan and Shaukat [22] and Abdulahi *et al* [23]. The results of the physico-chemical property of the soil used for the experiment are given in Table 1.

Table 1: Physico-chemical properties of the uncontaminated soil

Soil Parameter	Result
pH	5.20 ± 0.00
Sand (%)	81.60 ± 0.00
Clay (%)	11.70 ± 0.10
Silt (%)	6.70 ± 0.10
C (%)	0.18 ± 0.00
Organic matter (%)	0.31 ± 0.00
N (%)	0.17 ± 0.00
P (mg/kg)	12.40 ± 1.00
Ca (cmol/kg)	14.30 ± 0.30
Mg (cmol/kg)	7.70 ± 0.40
Na (cmol/kg)	0.10 ± 0.40
K (cmol/kg)	0.40 ± 0.05
CEC (cmol/kg)	22.50 ± 0.60
Pb (mg/kg)	< 0.08
Cd (mg/kg)	< 0.01
Cu (mg/kg)	0.30 ± 0.00
Zn (mg/kg)	1.00 ± 0.10

The result of the physicochemical property of the soil indicates that the soil is sandy loam with high sand content of 81.60%. The soil is slightly acidic, very low in organic matter, exchangeable potassium (K) and Sodium (Na). The soil thus needs amendment if intended for agricultural purpose. The experimental soil contained low concentration of Cu (0.30mg/kg) and Zn (1.00mg/kg), while Cd and Pb were not detected in the soil sample hence the soil had to be spiked with the metal salts of the metals for thus study. The amount of total heavy metals in the experimental soils sample after contaminating with 0.3g of salts of Cd, Cu, Pb and Zn are given in Table 2.

Table 2: Concentration of heavy metal of the contaminated soil samples

Metals	Amounts (mg/kg)
Pb	298.90 ± 1.20
Cd	296.40 ± 1.00
Cu	300.10 ± 1.70
Zn	300.70 ± 1.00

The sequential extraction conducted on the amended contaminated soil samples revealed that the concentration of metals (Cd, Cu, Pb and Zn) were highest in the residual fraction. This shows that metals will not be easily available to plant to absorb. Metal species associated with organic, Fe-Mn oxide and residual fractions are not readily bioavailable and would not be expected to be released under natural conditions as they are tightly held and bound. Their release into the soil solution depends on strong depletion of minerals content of the soil, solution, decomposition and oxidation of organic matter.

Table 2: Amount of metals (mg/kg) in poultry dropping amended metal contaminated soils before planting

Amendment	Metal	Exchangeable	Carbonate	Fe-Mn oxide	Organic	Residual	Sum
0 %	Cd	34.70 ± 1.90	48.20 ± 2.10	67.40 ± 1.30	55.20 ± 1.70	73.80 ± 1.20	279.30
	Cu	53.30 ± 2.00	42.10 ± 0.00	56.80 ± 1.20	63.40 ± 1.80	72.30 ± 1.30	287.90
	Pb	42.20 ± 1.80	49.30 ± 1.20	57.60 ± 1.00	64.20 ± 1.00	70.10 ± 1.80	283.40
	Zn	43.40 ± 1.40	48.90 ± 1.30	54.30 ± 1.00	63.70 ± 1.60	81.00 ± 2.10	291.30
5%	Cd	35.30 ± 1.80	46.90 ± 1.90	67.70 ± 1.10	54.90 ± 2.10	73.60 ± 1.70	278.40
	Cu	51.80 ± 2.10	43.30 ± 1.90	56.10 ± 1.40	64.50 ± 1.80	71.90 ± 1.40	287.60
	Pb	41.80 ± 2.10	49.20 ± 1.90	57.90 ± 1.90	65.30 ± 2.10	69.70 ± 1.40	283.90
	Zn	44.80 ± 1.20	47.40 ± 1.90	56.80 ± 2.00	61.20 ± 1.70	80.90 ± 1.40	291.10
10 %	Cd	37.90 ± 2.00	47.80 ± 1.10	67.10 ± 1.80	53.70 ± 1.40	74.60 ± 1.00	281.10
	Cu	55.20 ± 1.00	42.90 ± 1.00	57.70 ± 1.50	64.10 ± 1.30	68.70 ± 1.90	288.60
	Pb	41.40 ± 1.70	49.90 ± 1.80	56.20 ± 2.00	64.70 ± 1.20	70.90 ± 2.10	283.10
	Zn	47.40 ± 3.10	52.10 ± 1.40	55.90 ± 1.90	62.70 ± 2.30	74.80 ± 3.00	292.90
20 %	Cd	35.80 ± 1.90	48.20 ± 1.10	66.40 ± 2.00	54.60 ± 1.20	75.80 ± 1.40	280.80
	Cu	48.70 ± 2.00	43.80 ± 1.00	56.60 ± 1.00	63.90 ±1.40	73.90 ± 1.70	287.90
	Pb	43.60 ± 1.80	51.30 ± 1.40	56.20 ± 2.00	63.70 ± 1.70	69.70 ± 2.10	284.50
	Zn	43.90 ± 0.00	51.70 ± 1.50	57.80 ± 2.10	66.20 ± 1.40	71.80 ± 2.60	291.40
30 %	Cd	37.40 ± 1.80	47.70 ± 2.00	66.90 ± 1.30	53.40 ± 1.70	75.00 ± 1.90	281.40
	Cu	52.40 ± 2.20	41.10 ± 2.10	58.30 ± 2.40	64.60 ± 1.80	72.40 ± 1.70	288.80
	Pb	43.90 ± 2.10	49.40 ± 1.50	57.80 ± 1.60	62.70 ± 1.50	70.50 ± 1.40	284.30
	Zn	45.30 ± 2.00	49.90 ± 1.80	56.20 ± 2.40	64.80 ± 2.70	77.10 ± 2.10	293.30

The mobility factors of the metal in the poultry dropping amended soils are shown in Fig 1. Mobility factor of a metal represent the amount of metal that is available to plant or amount of metal plant can absorb from a contaminated soil. From the graph, it was observed that the mobility factor of Cu was the highest in the 0, 5 and 10% amended soils while the mobility factor of Pb was the highest in the 20 and 30% amended soils. In all soil samples the mobility factor of Cd was the lowest indicating that the amount of Cd available for uptake by the sclerotia was the lowest.

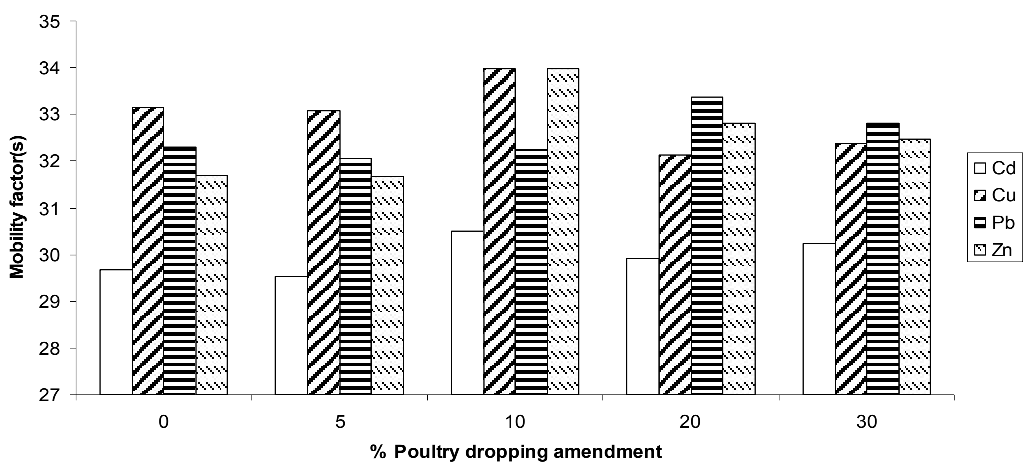

Fig 1: Mobility factors of metals in amended soils before planting

The amount of metal uptake by sclerotia from amended contaminated soils is shown in Fig 2. The graph revealed that the amount of Cu uptake by sclerotia after fifteen days of planting was the highest of all metals. The bioaccumulation order was Cu > Zn > Pb > Cd for the various amount of poultry dropping amended soil. Minimum and maximum concentration of Cu accumulation by the sclerotia was 10.50 and 41.9mg/kg respectively cadmium bioaccumulation in the sclerotia, samples ranged from 3.9 to 17.9mg/kg. It was also evident that as the percentage of amendment increased, the amount of metal taken up decreased.

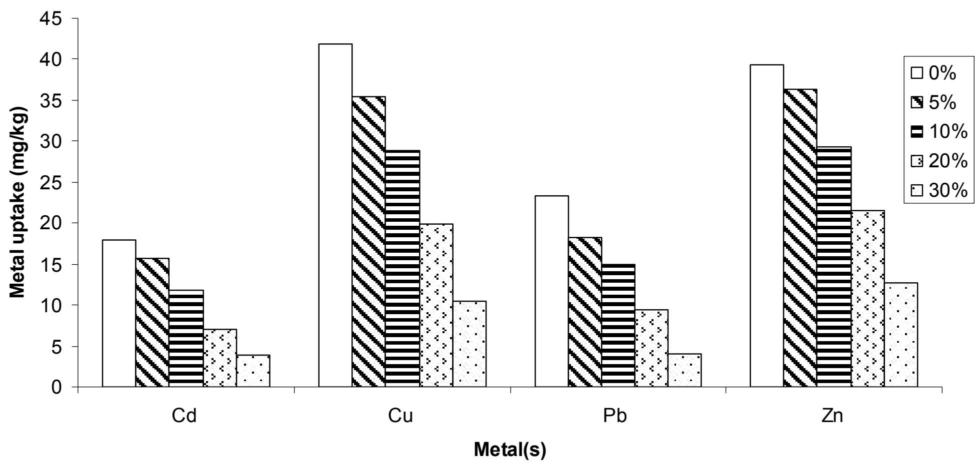

Fig 2: Metal uptake by sclerotia from amended contaminated soil

The result given in Table 3 showed that the concentration of metals in the non available fraction of the soil (Fe-mn oxide, Organic and residual fractions) was higher than the levels obtained for amended soils before planting. The result revealed that the amount of metals in the residual fraction decreases with an increase in
amendment indicating that the added poultry droppings immobilize the metal in the contaminate soil.

Table 3: Amount of metals (mg/kg) in poultry dropping amended metal contaminated soils after planting

Amendment	Metal	Exchangeable	Carbonate	Fe-Mn oxide	Organic	Residual	Sum
0 %	Cd	29.90 ± 5.30	41.50 ± 4.80	63.90 ± 4.90	52.80 ± 5.60	71.70 ± 6.30	266.40
	Cu	34.80 ± 4.20	41.60 ± 3.80	47.70 ± 3.40	51.20 ± 3.80	62.60 ± 4.30	237.90
	Pb	33.80 ± 4.60	41.90 ± 5.30	51.70 ± 4.00	58.60 ±3.60	67.70 ± 3.10	253.70
	Zn	31.70 ± 3.00	39.30 ± 2.10	44.80 ± 3.40	59.10 ± 4.00	72.80 ± 3.90	247.70
5%	Cd	31.60 ± 6.20	42.80 ± 4.70	64.80 ± 5.30	54.30 ± 4.80	72.90 ± 4.30	266.40
	Cu	31.80 ± 4.70	40.70 ± 3.30	48.60 ± 2.90	53.60 ± 3.20	64.70 ± 3.30	239.40
	Pb	34.20 ± 6.00	41.30 ± 4.70	53.20 ± 4.30	59.70 ± 5.20	68.60 ± 4.70	257.00
	Zn	29.20 ± 4.60	41.60 ± 3.00	44.90 ± 2.70	59.70 ± 3.20	71.40 ± 3.60	246.80
10 %	Cd	30.30 ± 4.80	39.60 ± 5.40	65.40 ± 6.30	53.30 ± 6.10	73.00 ± 3.80	261.60
	Cu	32.60 ± 3.10	41.30 ± 2.30	50.20 ± 3.40	54.80 ± 3.00	66.30 ± 4.40	245.20
	Pb	32.40 ± 4.80	44.20 ± 4.00	56.70 ± 5.30	63.80 ± 6.10	69.30 ± 5.00	266.40
	Zn	28.80 ± 3.70	42.70 ± 3.20	46.40 ± 3.90	61.10 ± 3.30	73.60 ± 3.90	252.60
20 %	Cd	25.30 ± 2.40	38.20 ± 4.80	67.90 ± 5.20	53.60 ± 4.90	77.40 ± 2.10	262.40
	Cu	27.60 ± 2.40	41.90 ± 4.40	52.30 ± 5.10	56.60 ± 3.80	71.80 ± 3.90	250.20
	Pb	29.70 ± 4.30	43.40 ± 4.00	57.70 ± 2.70	64.80 ± 3.80	75.40 ± 4.50	271.00
	Zn	25.40 ± 3.00	42.20 ± 3.60	48.20 ± 3.70	62.90 ± 4.10	82.70 ± 3.00	261.40
30 %	Cd	22.60 ± 3.60	37.80 ± 4.00	69.40 ± 3.70	54.90 ± 5.20	79.80 ± 4.80	264.50
	Cu	29.60 ± 4.00	41.40 ± 3.20	56.30 ± 4.20	59.70 ± 3.60	74.80 ± 3.10	261.80
	Pb	28.00 ± 5.40	41.90 ± 3.80	59.40 ± 4.20	66.30 ± 3.70	77.70 ± 6.40	273.30
	Zn	30.70 ± 2.90	41.40 ± 3.10	47.60 ± 3.80	63.80 ± 3.30	83.90 ± 4.20	267.40

The mobility factor of metals in amended contaminated soils after planting is given in Fig 3. The graph revealed that Cu has the highest potential mobility of all the heavy metals in the amended soil samples. The mobility factor of Cd was highest in the 5% amended soil and lowest in the 30% amended soil. The mobility factor of Cu decreased with increase in amount of poultry dropping added to the Cu contaminated soil. The mobility factors of Pb were same for the 0 and 5% amended and the factor then decreased with increase in amendment

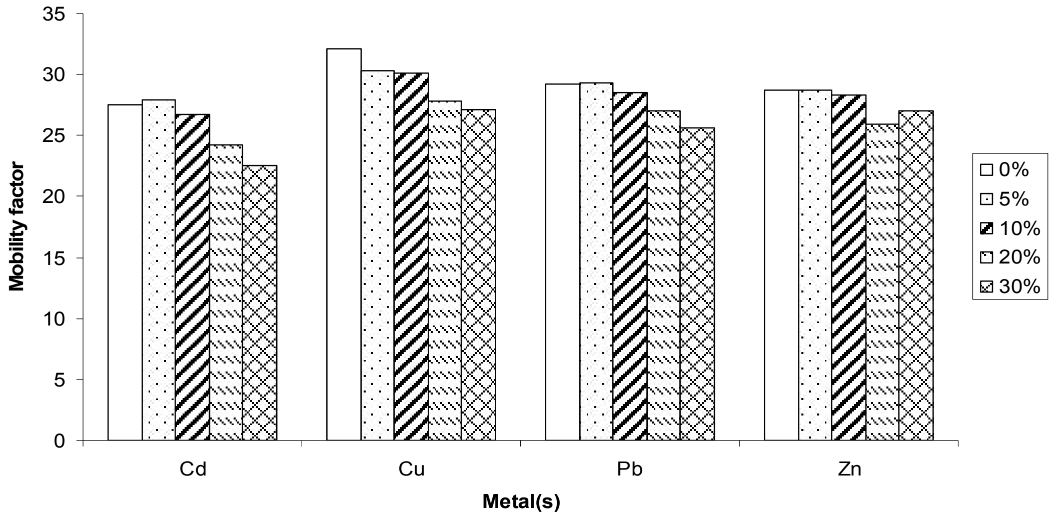

Fig 3: Mobility factor of metals in amended contaminated soils after palnting

CONCLUSION

The study which focused on the bioaccumulation potential of heavy metal by white rot fungi revealed the efficiency of the white rot fungi in remediating metal contaminated soil. The uptake of the metals by the fungi was also found to have a relationship with concentration amendment added to the metals contaminated soil. This fact is of particular importance to mushroom pickers and consumers.

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